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(54) **MULTIVALENT DENGUE VIRUS VACCINE**

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See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,723,130 A 3/1998 Hancock et al. 424/211.1
6,638,514 B1 * 10/2003 Eckels et al. 424/218.1

OTHER PUBLICATIONS

Trent, D. W., et al., "Genetic variation and microevolution of dengue 2 virus in Southeast Asia.", *VIROLOGY*, (Oct. 1989) 172(2): 523-535.*

Rico Hesse, R., et al., "Molecular evolution of dengue type 2 virus in Thailand.", *American Journal of Tropical Medicine and Hygiene*, (Jan. 1998) 58(1):96-101.*

Holmes, E. C. and S. S. Burch, "The causes and consequences of genetic variation in dengue virus.", *Trends in Microbiology*, (Feb. 2000) 8(2):74-77.*

Harrison, V. R., et al., "Virulence and immunogenicity of a temperature sensitive dengue 2 virus in lower primates.", *Infection and Immunity*, (Oct. 1977) 18(1):151-156.*

Kinney, R. M., et al., "Construction of Infectious cDNA clones for dengue 2 virus: strain 16681 and its attenuated vaccine derivative, strain PDK 53.", *VIROLOGY*, (Apr. 14, 1997) 230(2):300-308.*

Vaughn, D. W., et al., "Testing of a dengue 2 live-attenuated vaccine (strain 16681 PDK 53) in ten American volunteers.", *VACCINE*, (1996) 14(4):329-336.*

Edelman, R., et al., "A live attenuated dengue-1 vaccine candidate (45AZ5) passaged in primary dog kidney cell culture is attenuated and immunogenic for humans", *JID*, (1994) 170:1448-1455.*

Angsubhakorn, S., et al., "Dengue-3 (16562) PCMK 33 vaccine: neurovirulence, viremia, and immune responses in *Macaca fascicularis*", *Southeast Asian J. Trop. Med. Public Health*, (Sep. 1994) 25(3):554-559.*

Hoke, C. H., et al., "Preparation of an attenuated dengue 4 (34750 Carib) virus vaccine. II. Safety and immunogenicity in humans", *Am. J. Trop. Med. Hyg.*, (1990), 43(2):219-226.*

Botstein and Shortle, 1985, "Strategies and applications of in vitro mutagenesis", *Science*, vol. 229, No. 4719, pp. 1193-1201.

Clarke and Casals, 1958, "Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses", *Am. J. Trop. Med. Hyg.*, 7, 561-573.

Halstead et al., 1984, "Selection of attenuated Dengue 4 viruses by serial passage in primary kidney cells," *Am J. Trop. Med. Hyg.*, 33(4), pp. 654-665.

Halstead et al., 1984, "Selection of attenuated Dengue 4 viruses by serial passage in primary kidney cells," *Am J. Trop. Med. Hyg.*, 33(4), pp. 666-671.

Halstead et al., 1984, "Selection of attenuated Dengue 4 viruses by serial passage in primary kidney cells," *Am J. Trop. Med. Hyg.*, 33(4), pp. 672-678.

Halstead et al., 1984, "Selection of attenuated Dengue 4 viruses by serial passage in primary kidney cells," *Am J. Trop. Med. Hyg.*, 33(4), pp. 679-683.

Hayflick, 1988, "History of cell substrates used for human biologicals", *Symposium on Continuous Cell Lines as Substrates for Biologicals*, Arlington, Virginia, USA, pp. 11-26.

Hoke et al., 1990, "Preparation of an attenuated Dengue 4 virus vaccine", *Am J. Trop. Med. Hyg.*, 43(2), pp. 219-226.

Marchette, 1990, "Preparation of an attenuated Dengue 4 virus vaccine", *Am J. Trop. Med. Hyg.*, 43(2), pp. 212-218.

Mizrahi, ed., *Viral Vaccines*, "WHO Attitude to Viral Vaccines", Wiley-Liss, New York (1990), pp. 39-60.

Putnak et al, 1996, "Development of a purified, inactivated, Dengue-2 virus vaccine prototype in vero cells: immunogenicity and protection in mice and Rhesus monkeys," *J. Infectious Dis.*, 174, pp. 1176-1184.

(Continued)

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(57) **ABSTRACT**

The present invention provides vaccine compositions of attenuated dengue virus. More specifically, the attenuated virus is produced by serial passage in PDK cells. The invention also provides methods for stimulating the immune system of an individual to induce protection against all four dengue virus serotypes by administration of attenuated dengue-1, dengue-2, dengue-3, and dengue-4 virus.

13 Claims, 11 Drawing Sheets